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(Name and address of applicant, and in case of body corporate-place of incorporation)

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COHESIVE BIOPOLYMERS COMPRISING SULFATED POLYSACCHARIDES AND FIBRILLAR PROTEINS AND USE THEREOF FOR TISSUE REPAIR

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COHESIVE BIOPOLYMERS COMPRISING SULFATED POLYSACCHARIDES AND FIBRILLAR PROTEINS AND USE THEREOF FOR TISSUE REPAIR

FIELD OF THE INVENTION

The present invention relates to compositions comprising sulfated polysaccharides combined with fibrillar proteins, exemplified by dextran sulfate combined with gelatin, that form a cohesive biopolymer having unique physicochemical attributes useful as universal biomatrices or scaffolds for clinical applications including as implants for tissue engineering as well as in biotechnology. The matrices according to the present invention may be used clinically either per se or as a scaffold for a cell-bearing implant.

BACKGROUND OF THE INVENTION

Matrices useful for guided tissue regeneration and/or as biocompatible surfaces useful for tissue culture or tissue implants are well known in the art. These matrices may therefore be considered as substrates for cell growth either in vitro or in vivo. Suitable matrices for tissue growth and/or regeneration and/or implants include both biodegradable and biostable entities. Among the many candidates that may serve as useful matrices claimed to support tissue growth or regeneration, are included gels, foams, sheets, and numerous porous particulate structures fabricated at different densities and in different forms and shapes.

In many instances the matrix may advantageously be composed of biopolymers, including polypeptides or proteins, as well as various polysaccharides, including proteoglycans, sulfated polyglycans and the like. In addition, these biopolymers may be either selected or manipulated in ways that affect their physicochemical properties. For example, biopolymers may be cross-linked either enzymatically, chemically or by other means, thereby providing greater or lesser degrees of rigidity or susceptibility to degradation.

Among the manifold natural polymers which have been disclosed to be useful for tissue engineering or culture, one can enumerate various constituents of the

extracellular matrix including fibronectin, various types of collagen, and laminin, as well as keratin, fibrin and fibrinogen, hyaluronic acid, heparan sulfate, chondroitin sulfate and others.

US patents 5,955,438 and 4,971,954 disclose collagen-based matrices cross-linked by sugars, useful for tissue regeneration.

US patent 5,948,429 disclosing methods of making and using biopolymer foams comprising extracellular matrix particulates.

US 6,083,383 and 5,411,885 disclose fibrin or fibrinogen glue and methods for using same. US 5,279,825 and 5,173,295 disclose a method of enhancing the regeneration of injured nerves and adhesive pharmaceutical formulations comprising fibrin. US 4,642,120 discloses the use of fibrin or fibrinogen glue in promoting repair of defects of cartilage and bone.

US patents 6,124,265 and 6,110,487 disclose methods of making and cross-linking keratin-based films and sheets and of making porous keratin scaffolds and products of same.

Hyaluronic acid (HA) is a naturally occurring high molecular weight linear polymer belonging to the glycosaminoglycan family, composed of repeating units of glucuronic acid and N-acetyl glucosamine. HA readily forms hydrated gels which serve in vivo as space filling substance. The utility of hyaluronic acid as a beneficial component for supporting tissue growth is well established in the art, as exemplified in US 5,942,499 which discloses methods of promoting bone growth with hyaluronic acid and growth factors. US 5,128,326 and 5,783,691 disclose methods of producing and using cross-linked hyaluronans in promoting tissue repair and as reservoirs for bioactive agents including drugs or growth factors

Laminin (LN) is an adhesive glycoprotein of high molecular weight, which is known as a major cell matrix binding component. US patents 4,829,000 and 5,158,874 exemplify uses of gels or matrices comprising laminin.

WO 92/21354 discloses biocompatible anionic polymers that inhibit fibrosis, scar formation and surgical adhesions. Anionic polymers for use in the invention include but are not limited to natural proteoglycans, and the glycosaminoglycan (GAG) moieties of proteoglycans. The anionic polymers dextran sulfate and pentosan polysulfate are preferred, and according to a more preferred embodiment Dextran

Sulfate, preferably with a molecular weight of 40,000 to 500,000 Daltons in which the sulfur content is greater than about 10% by weight is used.

US 5,861,382 and US 6,020,323 disclose substances comprising carboxylated or sulfated oligo-saccharides in substantially pure form, and methods of using same for the regulation of cytokine activity in a host.

One of the present inventors has previously disclosed (WO 01/02030) a device with a constant perfusion system for maintenance of viable cells, tissues and composite implants. That disclosure further concerns a scaffold which is used as a growth supportive base for various cells and tissue explants comprising naturally derived connective tissue or skeletal tissue, cross-linked with one of the following: HA, proteoglycans, GAGs, chondroitin sulfates, heparan sulfates, heparins and dextran sulfates.

Cross-linking between macromolecules of the extra-cellular matrix may occur naturally (Laurent, 1964; Wang and Bozos, 1985). In vitro, heparin was reported to interact with natural occurring mammalian's proteins such as amyloid (Cohlbery et. al., 2002) or S-100 (Robinson et. al., 2002).

Certain specific combinations of polysaccharides and fibrillar proteins have been used to promote cell growth. For example, the non-sulfated polysaccharide chitosan has been combined with gelatin as a scaffold supporting chondrocyte growth and differentiation in vitro (Risbud M. et al. 2001); vascular cells responded in vitro and in vivo to chitosan and dextran sulfate (Chupa et.al., 2000); and auricular chondrocytes of elastic cartilage were shown to grow in hydrogels of collagen and alginate, a non-sulfated polysaccharide composed of polymannuronic acid de (Chalain et. al., 1999).

Dextran sulfate alone was found to act as antimicrobial agent (Christensen et al., 2001) and as a prophylaxis for peritoneal cancer metastasis (Hagiwara et al., 2000). It was also used as an antifoam agent of proteins (Ibanoglu et. al., 2001)

Nowhere in the background art is it taught or suggested that biopolymers comprising fibrillar proteins together with sulfated polysaccharides in general and dextran sulfate in particular are useful for clinical applications in vivo. Furthermore, the use of these combined scaffold matrices as an implant suitable for transplantation has never been disclosed.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a scaffold matrix that is biocompatible and affords a convenient environment for tissue repair. It is a further object of the present invention to provide a universal matrix scaffold suitable for many cell bearing implants which may conveniently be used either in vitro or in vivo. It is a further object of the present invention to provide a scaffold or gel matrix which is useful for clinical applications due to its unique attributes of fostering tissue regeneration.

The scaffold matrix may be fabricated in the shape of a gel, sleeve, cuff, sponge, membrane or any other shape useful as a scaffold for tissue repair.

These and other objects of the present invention are met by matrix gels comprising cohesive biopolymers of sulfated polysaccharides combined with gelatin or other fibrillar proteins. Preferred sulfated polysaccharides include dextran sulfate, chondroitin sulfate, heparan sulfate, heparin, keratan sulfate, dermatan sulfate, as well as algal polyglycan sulfates, or synthetic sulfated polysaccharides, as are known in the art. According to one currently more preferred embodiment exemplified herein dextran sulfate is combined with gelatin.

Though it is possible to use gelatin obtained from human collagen, more preferred are materials of non-human origin, due to safety concerns related to the use of collagens obtained from human sources. According to preferred embodiments it is possible to use porcine or bovine gelatin, though other mammalian species may also be used.

Though it is possible to use any dextran sulfate, more preferred are materials having high molecular weights. According to preferred embodiments it is advantageous to use bacterial dextran sulfate having a molecular weight of at least 500,000 Daltons, though other sources may also be used.

In order to provide other desired attributes, e.g. tensile strength, surface charge, density, porosity, ability to withstand suturing without tearing, etc., it is possible to add optional components either as a separate layer or interspersed or dispersed within the novel biopolymer of the invention.

According to additional features of the invention it may be advantageous to utilize cross-linking agents to alter or stabilize the attributes of the dextran sulfate-gelatin biopolymer. Cross-linking agents are known in the art and may include simple sugars such as pentoses or hexoses, or aldehydes such as glutaraldehyde, or synthetic cross-linkers if appropriate. It should be understood that while the interaction between the sulfated polysaccharides and the gelatin resulting in the cohesive biopolymer of the invention may be non-covalent, cross-linked copolymers may offer increased reproducibility as well as other improvement to the product.

According to a first aspect of the current invention we disclose an innovative material made of gelatin and dextran sulfate, having unique advantageous properties. The new copolymer allows the preparation of articles of various shapes, including but not limited to tubes and sheets suitable for the support of both a guide for peripheral nerve regeneration, a sleeve for coating or enclosing the spinal cord and a coating or envelope for a vascular and tracheal stent.

The novel biopolymers of the invention are useful in the fabrication of medical devices, the form or shape of these devices depending on intended use. The method for fabrication these devices may vary widely depending on the intended use.

These biopolymers are suited for use as fibers which fibers can be fabricated by conventional processes such as dry extrusion, gel extrusion, melt extrusion, solution extrusion or spinning extrusion, spraying of nanofibrils with or without an electromagnetic field, or by combination of these processes. The fibers can then be dried and spooled onto spools. The fibers can be woven, knitted, bundled or braided into complex form or constructs by methods known from industrial applications of textile manufacture.

The biopolymer of the invention is soluble in various aqueous or organic solvents and is suited for extrusion and co-extrusion with different components, organic or inorganic in nature and polymeric or otherwise, including multiple components, multilayered types of fiber as well as hollow fibers and tubes.

According to one currently more preferred embodiment, dextran sulfate is combined with gelatin, to provide scaffold matrices with unexpectedly advantageous chemical and physical properties, in addition to its biological properties of biocompatibility, controllable biodegradation rate, affinity for cultured cells, and

fostering cell growth. The novel cohesive biopolymer has physicochemical properties different from those of the uncombined raw materials, as can be evaluated by MRI analyses, infrared spectrum, elution from gel separation columns and other analytical tools known in the art.

According to a first embodiment of the invention, these matrices are useful *per se* as a biocompatible implant for guided tissue regeneration or tissue engineering. According to a further embodiment of the present invention these matrices are useful when they further comprise implants bearing cells to be transplanted to a site of injury or to ameliorate tissue impairment. According to a further embodiment of the present invention the matrices further comprise additional bioactive molecules to enhance tissue repair or regeneration.

Methods of using these cohesive biopolymers in vivo in clinical applications are disclosed, whereby the scaffold matrices according to the present invention may be used clinically either per se or as a scaffold for a cell bearing implant, alone or with additional layers of components. The cohesive biopolymers according to the present invention may advantageously be used as a substrate suitable for supporting cell selection, cell growth, cell propagation and differentiation in vitro as well as in vivo.

The cohesive biopolymers according to the invention comprise dextran sulfate in the range of about 30 % to about 70% (w/w) and gelatin in the range of about 30% to 70% (w/w). This range of ingredients provides scaffold with the desired properties in terms of flexibility and elasticity. Typically, the biopolymer of the invention may conveniently be formed by interaction of approximately equal amounts of dextran sulfate and gelatin

The present invention also provides for the addition of further active ingredients to matrices comprising dextran sulfate and gelatin, including but not limited to other proteins such as fibrin, albumin, collagen, elastin and lysozyme; one of the diverse polysaccharides proteoglycans and hyaluronate; cross-linkers such as factor XIII, lysyloxidase; anticoagulants; growth factors; antioxidants and the like. These optional additives may be incorporated in such a manner to provide for desired pharmacokinetic profiles. Within the scope of the present invention there are provided methods of using the dextran sulfate-gelatin biopolymer gels for sustained release of bioactive components in vivo. In other instances the additives may be incorporated in

such a manner to provide for short-lived optimal local concentrations of the bioactive molecules incorporated therein.

The physicochemical parameters of the cohesive biopolymer matrix may readily be optimized in accordance with the intended use of the scaffold, and methods are disclosed to provide guidance to the skilled artisan in optimization.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1, 2, 3. Different views of a nerve cuff made from a biopolymer of dextran sulfate combined with gelatin (GD biopolymer) according to the invention.

Figure 4 Gel filtration profiles on Sepharose CL-6B column of a) dextran sulfate vs. combined biopolymer of gelatin and dextran sulfate (GD biopolymer) b) gelatin vs. GD biopolymer.

Figure 5 Nuclear Magnetic Resonance spectra of a) dextran sulfate; b) gelatin; and c) combined biopolymer of gelatin and dextran sulfate.

Figure 6 Infrared spectra of a) dextran sulfate; b) gelatin; and c) combined biopolymer of gelatin and dextran sulfate.

Figure 7 Swelling degree of GD membranes in distilled water before and after cross-linking.

Figure 8 Swelling degree of GD membranes in simulated saliva solution before and after cross-linking.

Figure 9 Degradation rate of GD-membranes cross-linked with ribose over time.

Figure 10 Porosity of dry GD biopolymer membrane, as shown by scanning electron microscopy.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Sulfated polysaccharide-gelatin copolymers

In order to mimic the matrices of common connective tissues we have selected polymers simulating the two main constituents namely collagens and glycosaminoglycans. According to the currently most preferred embodiment gelatin is used to mimic the collagen, and dextran sulfate is used to simulate the glycosamino-glycans.

The collagen-based biological materials, their derivatives, and other biocompatible polymers have shown great promise in the area of tissue engineering. For this purpose we developed the new Gelatin-Dextran sulfate (GD) ionic complex that can be useful for guiding regeneration and as cell carrier in various fields. Dextran is a glucose biopolymer and dextran sulfate is a polysaccharide, composed of sulfated glucose as the repeating units, this sulfated polymer of bacterial origin best simulates the glycosaminoglycans.

The GD cohesive biopolymer is easily sterilized and stored at room temperature, capable of large scale production and moldable into various shapes. For example, we have created a tube, that designed to help restore function to patients with peripheral nerve injures (whole sectional loss) by acting as a bridge for guiding the nerve regeneration as exemplified below (also see Figures 1, 2, 3).

The biopolymer is useful in the fabrication of medical devices, the form or shape of these devices depending on their intended use. The method for fabrication of these devices may vary widely depending on the intended use.

The biopolymer is suited for use as fibers which fibers can be fabricated by conventional processes such as dry extrusion, gel extrusion, melt extrusion, solution extrusion or spinning extrusion or by combination of these processes. The fibers can then be dried and spooled onto spools. The fibers can be woven, knitted, bundled or braided into complex form or constructs by methods known from industrial applications of textile manufacture.

The biopolymer is suited for extrusion and co-extrusion with different components, organic or inorganic in nature and polymeric or otherwise, including multiple components, multilayered types of fiber as well as hollow fibers and tubes.

By using suitable methods as are known in the art it is possible to optimize this material for biocompatibility, cytotoxicity aspects, and other desired parameters including rate of biodegradability, tensile strength of fibers, flexibility of sheets and tubes, porosity, etc.

For controlling the biodegradation rate of the cohesive biopolymer products (i.e., to increase or decrease their biodegradability) it is possible to add a polymerizable macromolecule with known biocompatibility and known degradation time, exemplified but not limited to collagen, polyurethane, polyglycolic/polylactic

acids, trimethylene carbonate, among others. The improved material with for example incorporated carbonate and/or dioxanone linkages are selected to improve various properties of the material, particularly increasing viscosity, viscoelasticity and retention time, while prolonging yet preserving biodegradability.

Furthermore, we provide methods for allowing the presence of pores within the biopolymer material and for determining the preferred pore sizes. Pores may be desirable in relation to stimulation of cell adhesion, growth, and differentiation, and in the converse intactness may be needed for certain applications such as for formation of a tracheal stent.

The following examples are intended to illustrate the principles of the invention and are to be construed in a non-limitative manner.

Example 1: Manufacture of the GD Biopolymer

Porcine skin gelatin is diluted to 20 mg/ml with a buffer, containing 0.5 mM magnesium sulfate, 1.5 mM calcium chloride, 150 mM sodium chloride, 5 mM sodium or potassium phosphate, pH 7.4, serving as a standard balanced salt solution. The gelatin solution is stored at 70°C to prevent fibril formation.

Dextran sulfate is diluted to 20 mg/ml with the above buffer, with or without 2mM zinc chloride. The pH then is finely adjusted to pH 7.4 using 5% ammonium hydroxide.

The dextran sulfate solution is poured into a gelatin solution and incubated at 80°C, accompanied by continuous gentle mixing to allow formation of a homogenous mixture, and then the polymerization is initiated by addition 1M acetic acid, in an amount that adjusts the pH to 4.0

After 0.5-1 min. the reaction mixture is poured into methanol/ethanol (1/1 volume ratio) to which is added a small amount of ammonium hydroxide (5N), which results in the precipitation of the newly formed polymer while adjusting the pH to a value around a physiological or neutral pH 7.0.

The unpolymerized molecules remain soluble, while the cohesive biopolymer is removed from liquid as a precipitate. The final polymer has a high viscosity, almost

semisolid, with a high wet tensile strength around 70-75 MPa and high resistance to mechanical cutting (e.g., by a surgical suture of 20N).

The biopolymer can further be cross-linked by thermal treatment or by chemical agents, for example, acetone, ethyl-3(3-dimethyl amino) propyl carbodiimide (EDC) oxidizing agents that are capable of forming active groups like aldehydes. For example sodium periodate is capable of forming aldehydes readily reactive with free amino group followed by reduction with sodium borohydride.

The biopolymer is useful in the fabrication of medical devices, the form or shape of these devices depending on intended use. The method for fabrication these devices may vary widely depending on the use.

The biopolymer is suited for use as fibers, which can be fabricated by conventional processes such as dry extrusion, gel extrusion, melt extrusion, solution extrusion or spinning extrusion or by combination of these processes. The fibers can then be dried and spooled onto spools. The fibers can be woven, knitted, bundled or braided into complex form or constructs by methods known from industrial applications of textile manufacture.

The biopolymer is suited for extrusion and co-extrusion with different components, organic or inorganic in nature and polymeric or otherwise, including multiple components, multilayered types of fiber as well as hollow fibers and tubes.

<u>Example 2: Forming the GD-tube</u>

Briefly, a coprecipitate of type A gelatin derived from porcine skin (Sigma) and dextran sulfate of MW 500,000D(Sigma) was compression molded to yield a dense sheet, membrane or tube. Then the items were cross-linked by thermal treatment at 130°C for 20h and/or by simple monomeric sugars (e.g., ribose, glucose, etc.).

<u>Example 3: Testing the GD tube to serve as a stent-sleeve, or coating</u>

A GD-Tube in the length of 5 mm with a diameter of 2 mm was stretched over a balloon carrying a coronary stent. The balloon was inflated to 16 atmospheres with water. The sleeve remained intact under two inflation cycles of 16 atmospheres. This ability of the cohesive biopolymer for stretching displays its potential for serving as stent-sleeve to lower restenosis and thrombosis rates after angioplasty.

Example 4: Use of the GD tube for enclosing neuronal implant

The methodologies for the maintenance, growth and differentiation of neuronal cultures are known to be most sophisticated. Therefore, an extracellular matrix (ECM) milieu that mimics the in vivo substrate and requirements of neuronal cells is most desirable.

Tissue culture methods have gained attention as a substitute for the use of *in vivo* animal models. One direction was devoted to the creation and simulation *in vitro* of the *in vivo* environment, nature and composition of the extracellular matrix (ECM) for the cultured cells or explants. As disclosed previously by one of the present inventors (WO 02/39948), two major components, namely Hyaluronic acid (HA) and Laminin (LN), have emerged as essential candidates specially for neuronal and glial cell cultures.

The combination of HA and LN into one viscous adhesive gel (HA-LN gel, also referred to herein as NVR-N-gel) has provided a biomatrix for growing neuronal cells and explants that derived from both the central and the peripheral nervous systems. The combination of HA and LN, which are major components of the ECM have been introduced by the inventors as substrates for growing neuronal cells and explants derived from both the central and peripheral nervous systems.

It was disclosed previously (WO 02/39948) that in addition to providing a useful substrate matrix for a broad range of cell types in vitro, the HA-LN gel serves as a highly advantageous biocompatible implant and as delivery vehicle for transplantation.

Nevertheless, it turns out that in order to improve the mechanical properties it is desirable to enclose the HA-LN gels in a more rigid scaffold prior to implantation into a patient. As shown in the following protocol this is achieved by use of the dextran sulfate-gelatin cohesive biopolymer of the present invention as a scaffold enclosing the HA-LN gel implant, the latter with our without cells.

The NVR guiding tube (denoted herein as GD tube) will be filled with the NVR-N-Gel with or without cells.

Example 5: Characterization of the GD biopolymer

The GD biopolymer is produced by thermo-chemical processing of two simple polymeric molecules: dextran sulfate and gelatin. A series of tests were performed to compare the original raw materials and the new formed polymers, as described herein below:

Gel Filtration Chromatography (GFC)

Gel filtration chromatography (GFC) profile of substances depends on the molecular weight as well as the 3-D shape of the molecule. Ten mg of polymeric substance dissolved in double distilled water was placed on the column of Sepharose CL-6B and eluted with double distilled water. The excluded (void) volume was determined employing dextran blue of $2x10^6$ daltons, eluted in tube # 7 (11.5 ml) 1.5 ml/tube.

Polysaccharides of $1x10^6$ and up were excluded. Sugars were followed by the phenol method for neutral sugars (1ml sample+1ml 5% phenol solution + 5ml of concentrated sulfuric acid). The orange color developed was read in a spectro-photometer at a wavelength of 490 nm. As shown in Figure 4, the chromatogram of dextran sulfate alone (Fig.4a) and the chromatogram of the novel GD biopolymer (Fig.4c) are notably different.

Similarly, gel filtration chromatography was performed to compare gelatin alone and the GD biopolymer.

Ten mg of polymeric substance dissolved in 0.5 ml of DMSO was placed on Sepharose CL-6B column. Elution was performed with a 10% DMSO solution, collecting fractions of 1.5ml each. Proteins having molecular weights of $4x10^6$ daltons and above were eluted at the void volume. Proteins were detected by spectrophotometer at a wavelength of 280 nm. Again, the chromatogram profile of gelatin (Fig. 4b) is distinctly different from the profile of the biopolymer (Fig. 4c).

Nuclear Magnetic Resonance spectroscopy (NMR)

Figure 5 a-c describe NMR analyses of gelatin, dextran sulfate and the novel GD biopolymer. The results clearly show that the new biopolymer is distinguished from the original raw materials.

Infra red spectrometry

Figure 6 shows the infrared spectra of gelatin and dextran sulfate as raw materials in comparison with the spectrum of the GD biopolymer.

A gross analysis of the spectrum, showing that the spectrum of the GD biopolymer differ from those of the raw material molecules, suggests the formation of a new polymeric substance, as was shown by the results of the other tests described above.

Example 6: Characterization of GD biopolymers with different degrees of cross-linking

The degradation rate of the polymer can be controlled by the extent and type of the cross-linking between the polymer molecules. The method of the present invention, reacting the biopolymer with reducing sugars, resulted in intensive cross-linking of the biopolymer molecules. Comparing a full hand of cross-linking agents showed pentose monosaccharide ribose as the best agent. The degree of cross-linking is controllable by the sugar concentration, temperature and the length of the reaction.

Comparing the GD properties before and after cross-linking examined the influence of the cross-linking degree on the properties of the GD biopolymer.

Swelling test

The membranes swelling studies were conducted using two media, namely, distilled water and simulated saliva solution. Each sample of membrane (surface area $40mm^2$) was dried by vacuum for 4h, weighed and placed in a pre-weighed stainless steel wire mesh with sieve openings of approximately 200µm. The mesh sieve with the film sample was submerged into 25ml medium placed in a plastic beaker. Increase in weight of the membranes was determined at successive time intervals until a constant weight was obtained. Each measurement was repeated three times. The degree of swelling was calculated using the following parameters:

$$\frac{W_t - W_0}{W_0} \times 100\%$$

Where W_t is the weight of the membrane at time t; and W_0 is the weight of membrane at time zero.

The samples were tested before cross-linking, after cross-linking by dehydrothermal treatment and after cross-linking by a combination of dehydrothermal treatment and sugars.

Figures 7 and 8 depict the degree of swelling of the GD membranes in distilled water and simulated saliva solution, respectively, before and after cross-linking. The degree of swelling was higher in distilled water compared to saliva solution. This finding suggests that ionic strength and pH play an important role in affecting the swelling of the membranes. The rate of swelling for the GD membranes before cross-linking was higher compare to membranes after thermal cross-linking and significantly lower for membranes after the combination of thermal treatment with cross-linking by sugars. These data indicate that both the thermal method and the sugars cross-linking decrease the rate of water uptake and hydration, as a result of increasing the degree of cross-linking.

In vitro biodegradation

Gelatin-dextran sulfate-membranes (GD membranes) were prepared and cross-linked by ribose. Samples, prepared for testing, were dried by vacuum for 4 h, weighed and fully immersed in the physiological solution, supplemented with 20% fetal bovine serum at 37°C for the specified period of time. At each specified time period throughout the duration of the incubation time, the solution was replaced and samples were removed, dried and weighed. By measuring the weight change during the 30 days duration of the assay, the degradation rate was calculated. Figure 9 shows that the ribose cross-linked preparations are degraded at a rate of 2-2.5% per day.

Example 7: Porosity of the GD biopolymer

The structure of a dry GD membrane was examined by Scanning Electron Microscope (SEM), before and after incubation of the membrane in neuronal cell culture medium for 24 days. As shown in Fig. 10, the dry membrane appears as a continuous dense solid with a randomly porous structural; the size of the pores was 20-70 μm.

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CLAIMS

1. A biocompatible matrix comprising a cohesive biopolymer of a fibrillar protein and a sulfated polysaccharide.

2. The matrix of claim 1 wherein the protein is selected from the group consisting of collagen, elastin, fibrin, albumin and gelatin.

3. The matrix of claim 2 wherein the protein is gelatin.

4. The matrix of claim 1 wherein the sulfated polysaccharide is selected from the group consisting of dextran sulfate, chondroitin sulfate, heparin, heparan sulfate, keratan sulfate, dermatan sulfate, algal sulfated polymer, or a synthetic sulfated polysaccharide.

5. The matrix of claim 4 wherein the sulfated polysaccharide is dextran sulfate.

6. The matrix of claim 1 wherein the cohesive biopolymer comprises gelatin and dextran sulfate.

7. The matrix of claim 5 comprising 30% to 70% of dextran sulfate.

8. The matrix of claim 5 comprising 30% to 70% of gelatin.

9. The matrix of claim 1 further comprising anticoagulants, adhesive molecules, growth factors, enzymes, antioxidants, antifibrotic substances, positively charged molecules, a peptide rich in positively charged amino acids, and nutritional elements.

10. The matrix of claim 1 further comprising cross-linking bridges formed by a monosaccharide, factor XIII, lysyloxidase, a carbodimide, an oxidizing agent.

11. The matrix of claim 8 wherein the cross linkers are selected from the group consisting of ribose, glucose, mannose, xylose.

12. The matrix of claim 1 further comprising a bioactive compound selected from the group consisting of a hormone, a growth factor, a proteolytic enzyme, an anti-fibrotic agent, a coagulative agent, an extracellular matrix component, an anti oxidant, a natural or synthetic polymer.

13. The cohesive biopolymer matrix of claim 1 wherein the matrix is formed into fibers, sheets, sponges, fabrics or tubes.

14. The cohesive biopolymer matrix of claim 6 wherein the matrix is formed into fibers, sheets, sponges, fabrics or tubes.

15. The cohesive biopolymer matrix of claim 1 wherein the gel is formed into a scaffold enclosing neuronal cells.

16. The cohesive biopolymer matrix of claim 6 wherein the gel is formed into a scaffold enclosing neuronal cells.

17. The coheseive biopolymer matrix of claim 16 wherein the sleeve further comprises hyaluronic acid-laminin gel within the scaffold enclosing neuronal cells.

18. The cohesive biopolymer matrix of claim 1 wherein the sleeve is formed into a scaffold enclosing a cell bearing gel creating an implant.

19. An implant comprising a matrix according to claim 1.

20. An implant comprising a matrix according to claim 6.

21. A method for preparing a biocompatible matrix to be implanted in a human or animal biological medium, which comprises:

providing a solution of a fibrillar protein;

selecting a sulfated polysaccharide solution;

combining the two solutions at acidic conditions to form a cohesive biopolymer;

elevating the pH to precipitate the biopolymer from the solution.

22. The method of preparing the biocompatible matrix of claim 21 which further comprises shaping the matrix.

23. The method of claim 21 which further comprises incorporating a bioactive substance into the biopolymer.

24. The method of claim 21 wherein the fibrillar protein is gelatin.

25. The method of claim 21 wherein the sulfated polysaccharide is dextran sulfate.

26. A kit for carrying out extemporaneously a method according to claim 18, the kit comprising at least one dose of each constituent solution necessary to obtain the gel which forms the biocompatible matrix.

27. A composition for sustained release of a bioactive substance comprising a bioactive substance within a cohesive biopolymer according to claim 1 or claim 6.

For the applicants:

Cynthia Webb

Cynthia Webb
Webb & associates
Patent Attorneys

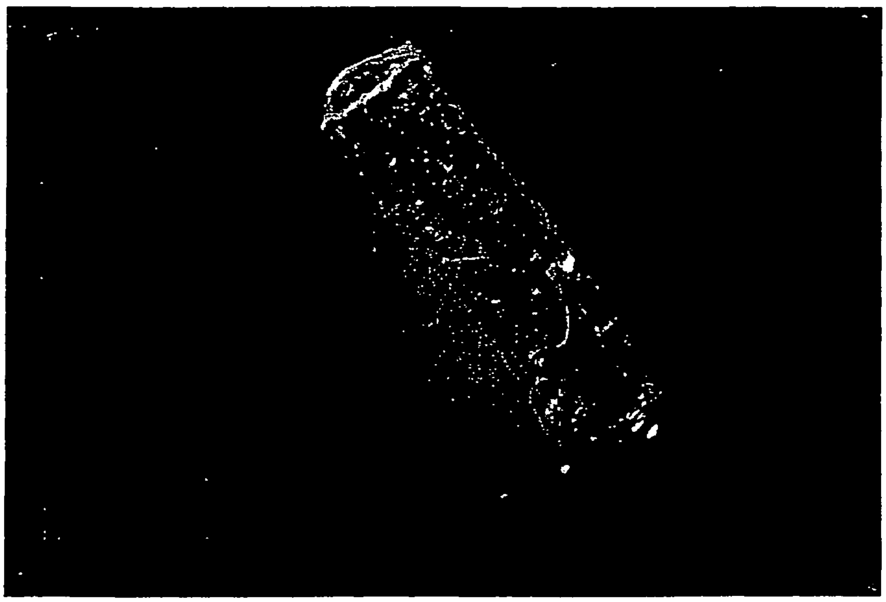


Figure 1

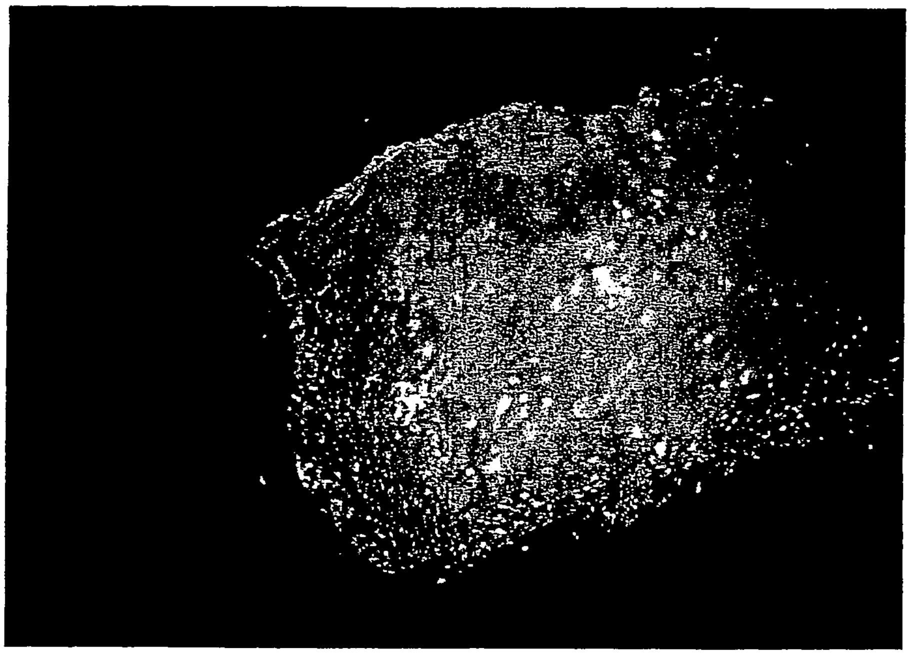


Figure 2

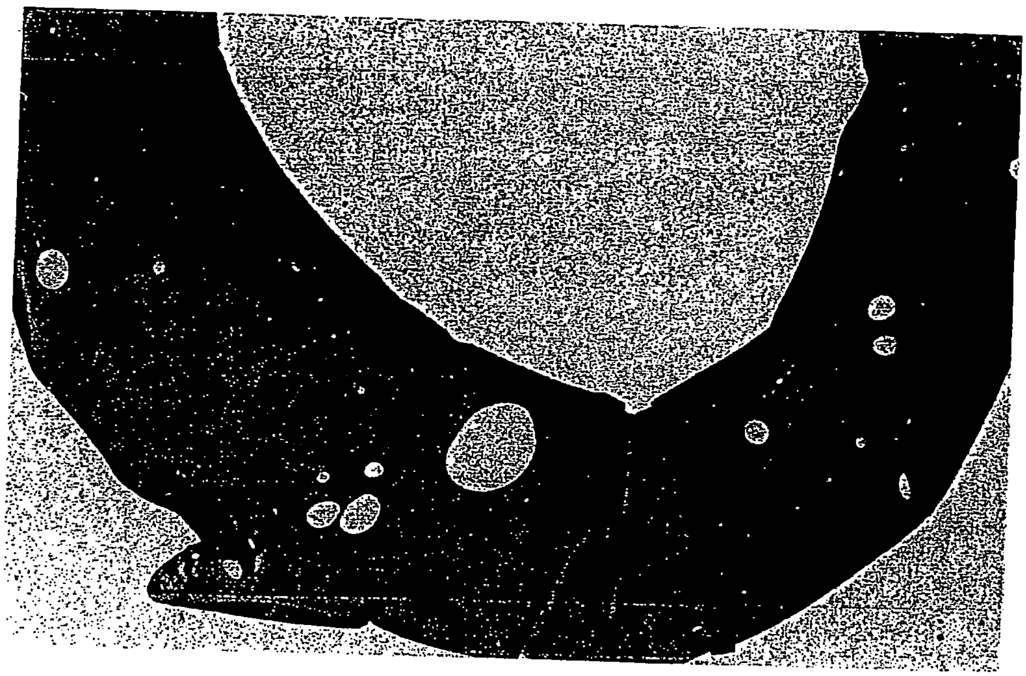


Figure 3

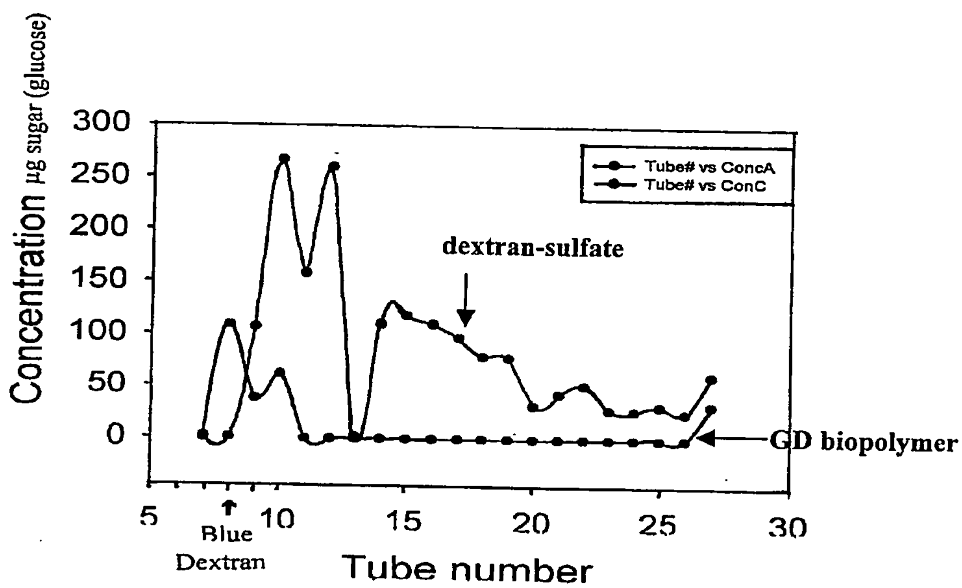

Concentration µg sugar (glucose)
300
250
200
150
100
50
0
Tube# vs ConcA
Tube# vs ConcC
dextran-sulfate
GD biopolymer
5
10
15
20
25
30
Blue
Dextran
Tube number

Figure 4a

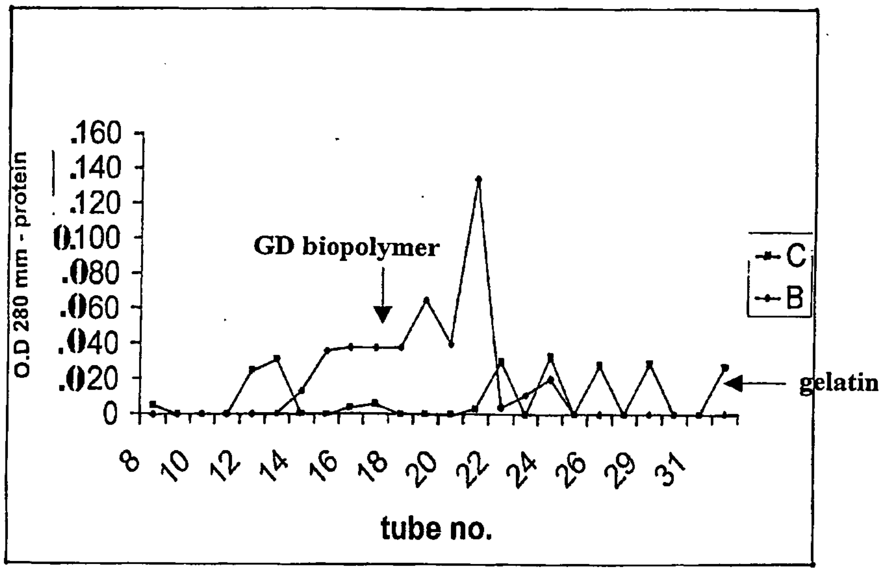

O.D 280 mm - protein
.160
.140
.120
0.100
.080
.060
.040
.020
0
GD biopolymer
C
B
gelatin
8
10
12
14
16
18
20
22
24
26
29
31
tube no.

Figure 4b

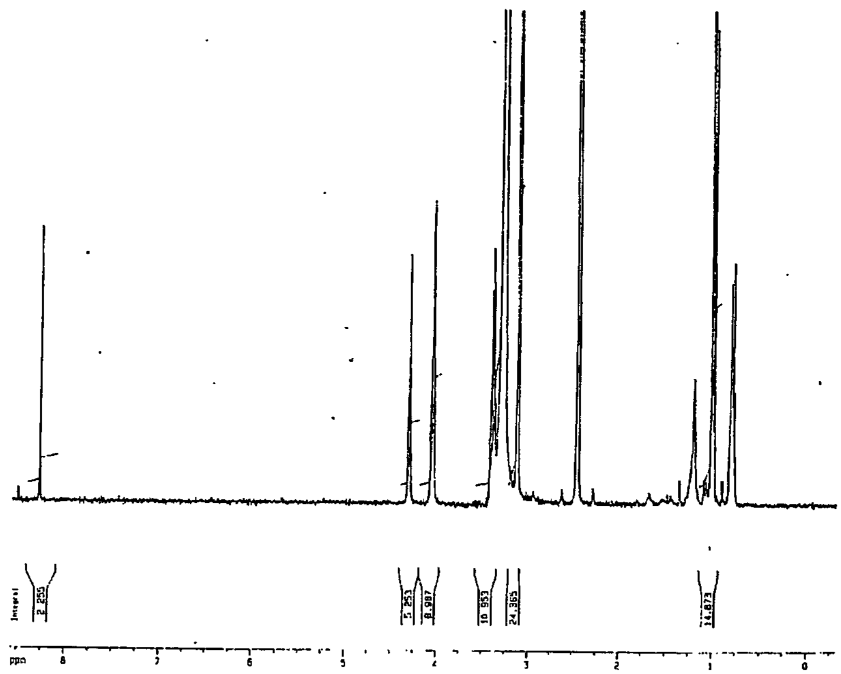


Figure 5a

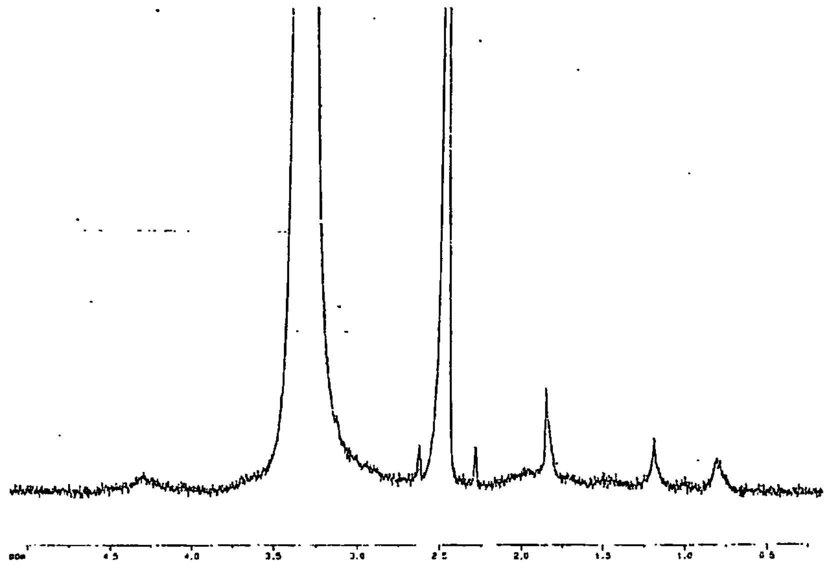


Figure 5b

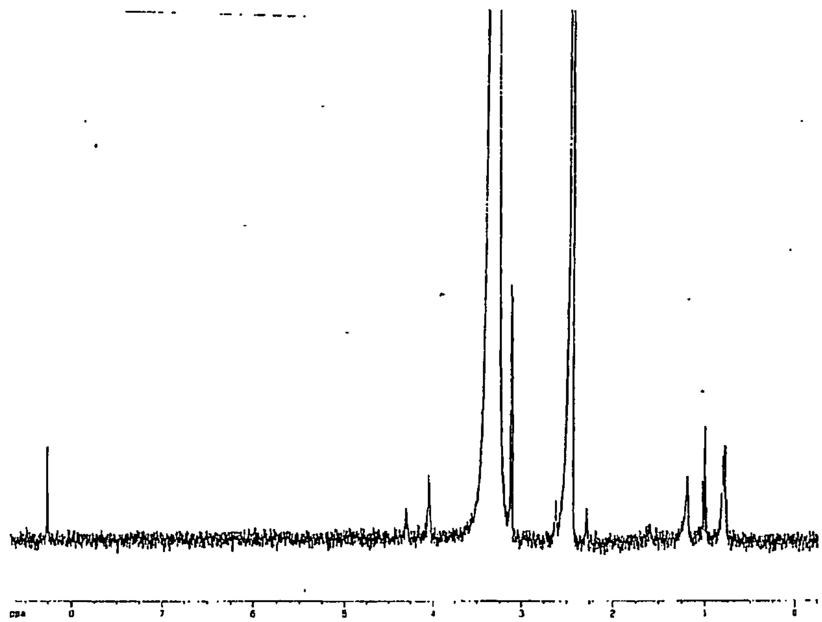
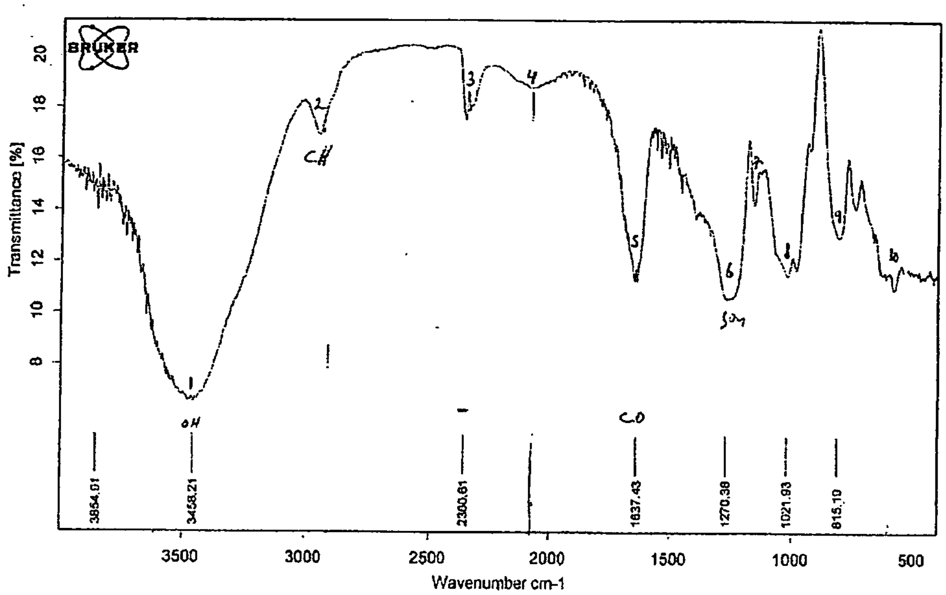


Figure 5c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Transmittance [%]
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18
16
14
12
10
8
CH
OH
CO
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3458.21
2300.61
1637.43
1270.38
1021.93
815.10
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

Figure 6a

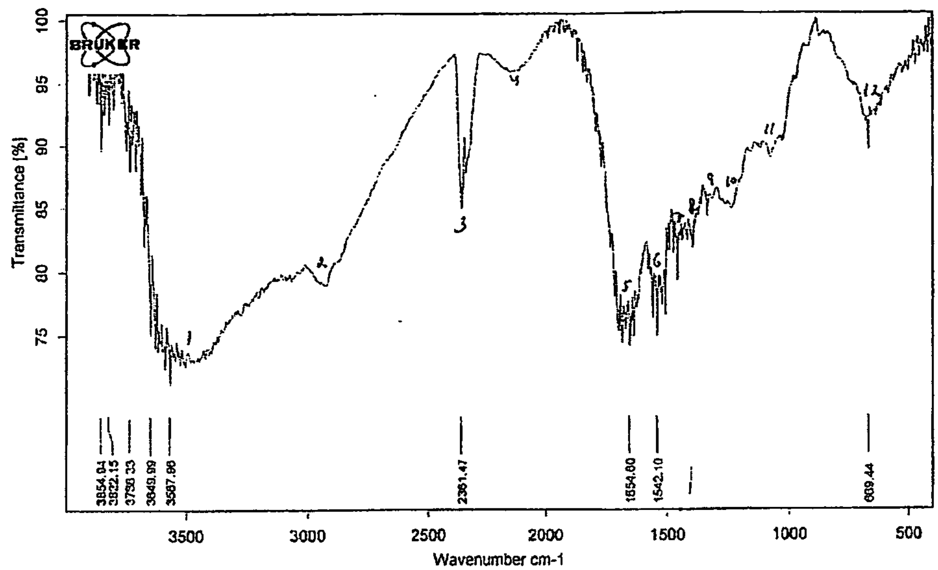
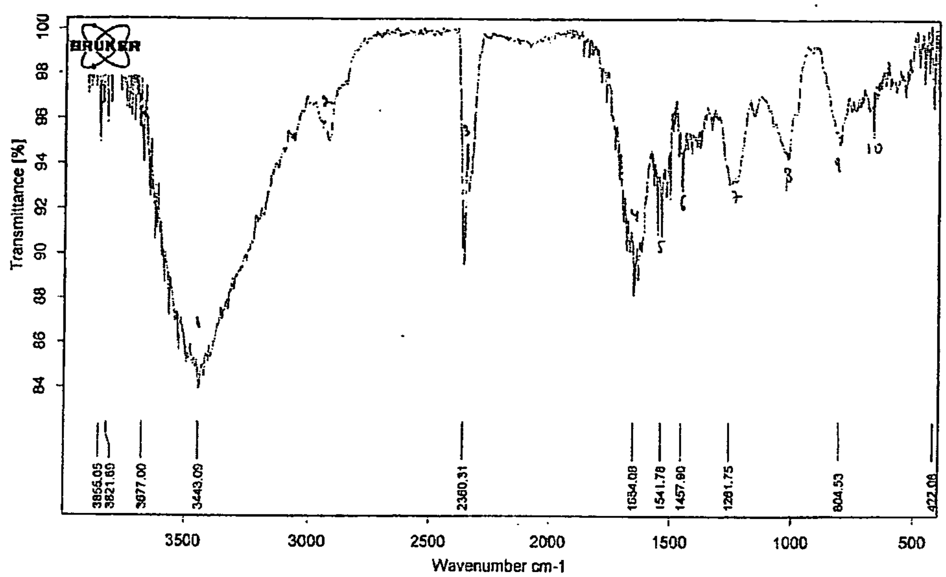

BRUKER
Transmittance [%]
100
95
90
85
80
75
3854.94
3822.15
3750.33
3649.99
3587.86
2361.47
1654.80
1542.10
639.44
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

Figure 6b


BRUKER
Transmittance [%]
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98
96
94
92
90
88
86
84
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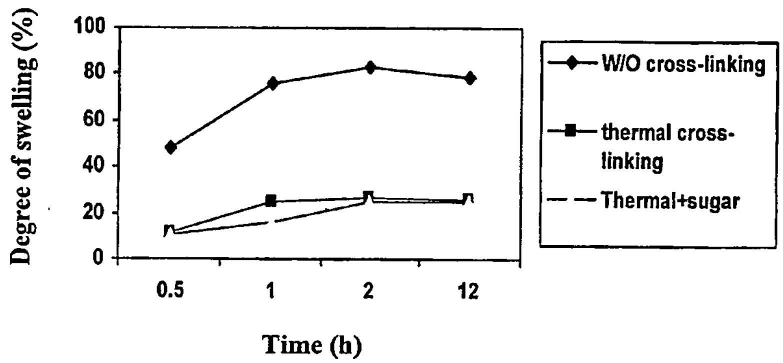
3821.69
3677.00
3443.09
2360.31
1634.08
1541.78
1457.80
1261.75
804.53
422.06
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

Figure 6c



Degree of swelling (%)
100
80
60
40
20
0
0.5
1
2
12
Time (h)
W/O cross-linking
thermal cross-linking
Thermal+sugar

Figure 7

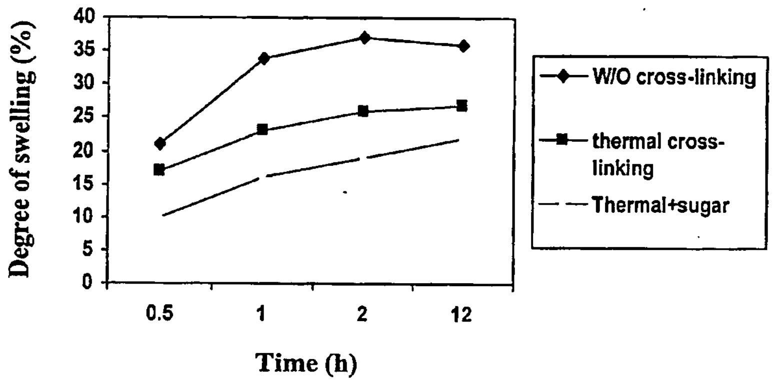

Degree of swelling (%)
40
35
30
25
20
15
10
5
0
0.5
1
2
12
Time (h)
W/O cross-linking
thermal cross-linking
Thermal+sugar

Figure 8

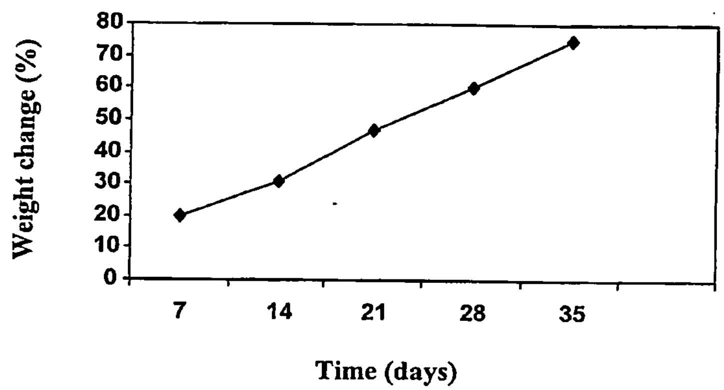


Figure 9

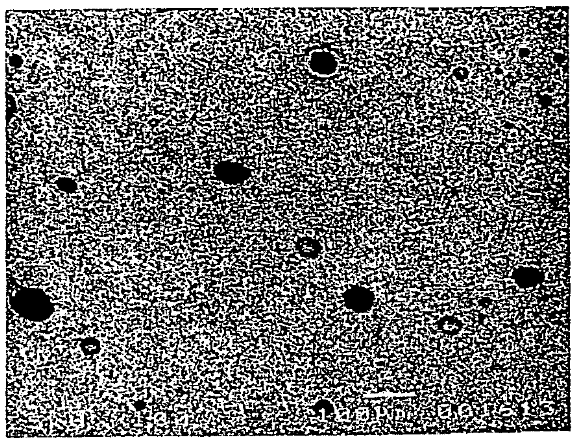


Figure 10